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Original article

Sufficient levels of 25-hydroxyvitamin D and protein intake required to increase muscle mass in sarcopenic older adults – The PROVIDE study

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SUMMARY

Background: Inadequate nutritional intake and altered response of aging muscles to anabolic stimuli from nutrients contribute to the development of sarcopenia. Nutritional interventions show inconsistent results in sarcopenic older adults, which might be influenced by their basal nutritional status.
Objective: To test if baseline serum 25-hydroxyvitamin D (25(OH)D) concentrations and dietary protein intake influenced changes in muscle mass and function in older adults who received nutritional intervention.
Methods and design: Post-hoc analysis was performed in the PROVIDE study that was a randomized controlled, double blind trial among 380 sarcopenic older adults. This study showed that those who received a vitamin D and leucine-enriched whey protein medical nutrition drink for 13 weeks gained more appendicular muscle mass (aMM), and improved lower-extremity function as assessed by the chair stand test compared with controls. To define low and high groups, a baseline serum concentration of 50 nmol/L 25(OH)D and baseline dietary protein intake of 1.0 g/kg/d were used as cut offs.
Results: At baseline, participants with lower 25(OH)D concentrations showed lower muscle mass, strength and function compared with participants with a high 25(OH)D, while the group with lower protein intake (g/kg/day) had more muscle mass at baseline compared with the participants with higher protein intake. Participants with higher baseline 25(OH)D concentrations and dietary protein intake had, independent of other determinants, greater gain in appendicular muscle mass, skeletal muscle index

Abbreviations: 25(OH)D, Serum 25-hydroxyvitamin D; aMM, Appendicular Muscle Mass; BW, Body weight; GDS, Geriatric Depression Scale; MNA-SF, Mini Nutritional Assessment-Short Form (MNA-SF®); MMSE, Mini Mental State Examination; PASE, Physical Activity Scale for the Elderly; SMI, Skeletal Muscle Mass Index (appendicular muscle mass /height^2); SPPB, Short Physical Performance Battery.

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(aMM/h^2), and relative appendicular muscle mass (aMM/body weight × 100%) in response to the nutritional intervention. There was no effect modification of baseline 25(OH)D status or protein intake on change in chair-stand test.

Conclusions: Sufficient baseline levels of 25(OH)D and protein intake may be required to increase muscle mass as a result of intervention with a vitamin D and protein supplement in sarcopenic older adults. This suggests that current cut-offs in the recommendations for vitamin D and protein intake could be considered the "minimum" for adults with sarcopenia to respond adequately to nutrition strategies aimed at attenuating muscle loss.

1. Introduction

Sarcopenia, the geriatric syndrome characterized by low muscle mass, strength, and function, will become increasingly prevalent as the global population ages. This syndrome places considerable stress on health care systems since it is implicated with impaired outcomes in chronic disease [1], as well as higher rates of hospitalization and nursing home admissions [2]. Inadequate nutritional intake and altered response of aging muscles to anabolic stimuli from meals contribute to the multifactorial pathogenesis of sarcopenia. In particular, inadequate intake of high quality protein including essential amino acids such as leucine and low 25-hydroxyvitamin D (25(OH)D) serum levels in older adults are potentially modifiable risk factors for sarcopenia [3–5].

Recent long-term nutrition intervention studies aimed at improving muscle mass, strength and function, however, have shown inconsistent results in sarcopenic and frail older adults [6,7]. The composition of the nutritional supplements, the amount and source of protein and amino acids, fat, carbohydrates and micronutrients such as vitamin D varied among the interventions. Moreover, variations in the health condition of the study populations, presence of multimorbidity, physical activity level, and nutritional status may have influenced the outcomes.

As a result of these heterogeneous findings, we hypothesized that baseline nutritional status could influence the efficacy of vitamin D and protein interventions. To test this hypothesis, we used the data from the PROVIDE study, in which sarcopenic older adults were randomized to either a vitamin D and leucine-enriched whey protein supplement or isocaloric control [8].

2. Materials and methods

2.1. Study design and participants

The PROVIDE study was a 13-week, multi-center, randomized, controlled, double blind, two parallel-group study among older adults with sarcopenia. Detailed information of the trial (registered under the Dutch trials register with the identifier NTR2329) has been published previously [8]. In brief, community-dwelling adults over 65 years were recruited from 18 study centers in Europe, and were eligible when presenting mild to moderate limitations in physical function (Short Physical Performance Battery (SPPB) score 4–9), and low skeletal muscle mass (≤37% (men) and ≤28% (women)) using bioelectric impedance analysis (BIA 101 Akern, Florence, Italy). Those who received the vitamin D and leucine-enriched whey protein medical nutrition drink gained more appendicular muscle mass (aMM), and improved lower-extremity function as assessed by the chair stand test, compared with controls (8).

Participants were randomized to receive either the intervention or an iso-caloric control product twice daily. The intervention product contained per serving 20 g whey protein, 3 g total leucine, 9 g carbohydrates, 3 g fat, 800 IU vitamin D and a mixture of vitamins, minerals and fibers, and the iso-caloric control drink contained only carbohydrates, fat and some trace elements.

Blinded and trained research staff collected information about the baseline characteristics via a questionnaire and assessed the outcomes during the study visits week 7 and 13. Self-reported amount of physical activity was assessed using the European version of the Physical Activity Scale for the Elderly (PASE). Health-related quality of life was determined using the EQ-5D, both as an index (0–1) and as a visual analogue scale (0–100). Cognitive function was measured using the Mini Mental State Examination (MMSE, 0–30) and cognitive impairment; i.e., MMSE <25 was an exclusion criterion. The Geriatric Depression Scale (GDS, 0–15 points) was used to assess potential depression symptoms. Finally, the Mini Nutritional Assessment-Short Form (MNA-SF®) was used to evaluate participants' nutritional status. The total score of the six questions (0–14 points) indicated whether the participant was well-nourished (12–14 points), at risk for malnutrition (8–11 points) or malnourished (0–7 points).

2.2. Muscle related outcomes

Appendicular muscle mass (aMM) was measured at baseline and week 13 using Dual energy x-ray absorptiometry (DXA, different models from Hologic, Bedford, USA; and Lunar, Fairfield, USA). Raw DXA data were centrally analyzed at the Vrije Universiteit Brussel, Brussels, Belgium, using a standardized protocol by the same researcher. Analyses were performed with and without correction for height^2 (skeletal muscle mass index, SMI: aMM/h^2) [9] or body weight (relative appendicular muscle mass: aMM/BW × 100%) [10].

The chair stand test measures the time required to rise five times from a chair without arm rests. It is one of the three components of the SPPB, along with gait speed and balance tests [2]. Maximum handgrip strength was calculated by taking the average of the highest measurement of two consecutive measures in each hand by using a hydraulic hand dynamometer (Jamar™, Preston, Jackson, Missouri, USA).

2.3. Serum 25-hydroxyvitamin D analysis

Analysis of serum 25(OH)D was performed at Reinier de Graaf Groep medical laboratory, Delft, the Netherlands using chemiluminescense micro-particulate immunoassay (Abbott Laboratories, Wiesbaden, Germany). The recovery of endogenous 25(OH)D both D3 and D2 species were 105% and 85%, respectively compared with a chromatography-based reference method. Serum 25(OH)D concentration was used as a dichotomous variable for most analyses with a cut-off of 50 nmol/L, which was similar to generally accepted threshold of serum 25(OH)D deficiency in older adults [11–13].

2.4. Dietary protein intake assessment

Dietary assessment was carried out at baseline and week 13 using 3-day food intake records for two days during the week and one day during the weekend. Additional energy and protein intakes from the supplements were added to the habitual 3-day intakes to estimate total intakes. Baseline protein intake was expressed as gram protein per kg of body weight per day. Baseline protein intake (g/kg/d) was used as a dichotomous variable for most analyses with a cut-off of 1.0 g/kg/day, representing the low-end of the most recent intake recommendations for healthy older adults [14,15].

2.5. Statistical analyses

We compared descriptive statistics to assess differences in characteristics at baseline between the low and high 25(OH)D and protein intake subgroups. Continuous data that were normally distributed were described with means and standard deviations and between-group comparisons were performed by two sample t-tests. Non-normally distributed data were described with medians and interquartile ranges and between-group comparisons were done using a Mann–Whitney test. Categorical variables were presented as percentages and either a Mann–Whitney test (ordinal data) or Fisher's exact (dichotomous data) was performed to test for significant differences between subgroups. ANCOVA models were used to test for a difference in the effect of the intervention on change in serum 25(OH)D concentrations between baseline 25(OH)D subgroups. These models were also used to test for a statistically significant difference of the effect of intervention group on appendicular muscle mass and chair-stand time between the 25(OH)D and protein intake subgroups (interaction effects). The ANCOVA models analyzed change from baseline of the endpoint in question and included the baseline value of the endpoint, age and sex as covariates. A separate sensitivity analysis was performed to test the difference between nutritional subgroups on the effect of intervention group on appendicular muscle mass and chair-stand time by men and women separately. A statistically significant interaction effect between treatment group and the subgroup indicates that the variable acted as an effect modifier. A separate ANCOVA was used with the PASE score or energy intake as a covariate to assess whether 25(OH)D and protein remained effect modifiers after adjusting for reported physical activity levels or dietary energy intake at baseline respectively.

In addition to the separate analyses with treatments by respectively baseline 25(OH)D concentration and baseline protein intake, combination of these two baseline factors in relation to treatment effects was tested in a combined ANCOVA model. The existing covariates (treatment group, age, sex) as well as baseline 25(OH)D, interaction of treatment group by baseline 25(OH)D, baseline protein, and interaction of treatment group by baseline protein were put as covariates into one model analyzing the intervention effects on change of aMM, SMI or relative appendicular muscle mass from baseline.

3. Results

The characteristics of a total of 380 participants of the PROVIDE study stratified by baseline 25(OH)D and baseline dietary protein intake are presented in Table 1, with body composition and muscle mass, strength and function characteristics separately for men and women. Participants with lower (<50 nmol/L) baseline 25(OH)D concentrations (n = 195) were less likely to be living independently, had slightly lower PASE, MMSE, and MNA scores, slightly higher GDS scores, and, predominantly in men, lower mean body weight, appendicular muscle mass, muscle strength and function compared with participants with higher (>50 nmol/L) baseline 25(OH)D concentrations (n = 179). Mean fat and lean body mass, dietary vitamin D intake, and protein intake were not different between the 25(OH)D groups.

There were no significant differences in demographic and clinical baseline characteristics between the two baseline protein intake subgroups, aside from more women in the higher protein intake subgroup (>1.0 g/kg/d). The participants in the higher protein intake (g/kg/day) subgroup had a lower mean weight, BMI, appendicular muscle mass and fat mass compared with the lower protein intake subgroup.

3.1. Effect modification of baseline 25-hydroxyvitamin D and protein intake on change in appendicular muscle mass and chair-stand time

The 25(OH)D concentrations increased significantly in the active group relative to baseline. Participants in the active group with lower baseline 25(OH)D concentrations had a greater increase in 25(OH)D (β active-control: 38.5 nmol/L (95% CI: 33.8–43.2)) than the higher 25(OH)D subgroup (β active-control: 25.3 nmol/L (95% CI: 20.4–30.3), overall p = <0.001). Participants in the high baseline 25(OH)D concentration group had significantly higher gain in appendicular muscle mass, SMI, and relative appendicular muscle mass compared with participants with 25(OH)D concentrations <50 nmol/L at baseline (Table 2). Adjustment for physical activity or dietary energy intake at baseline did not substantially change the results. There was no difference in chair-stand time between the low and high baseline 25(OH)D subgroups in their response to the intervention (Table 2).

Comparing the baseline dietary protein intake subgroups, a significantly higher gain of appendicular muscle mass, SMI, and relative appendicular muscle mass was observed in participants with a higher baseline protein intake. No effect of the intervention was found on change in chair-stand time dependent on baseline protein intake (Table 2).

The analysis of the combined effects on aMM change from baseline by both baseline 25(OH)D concentration and baseline protein intake resulted in a statistically significant interaction effect of treatment by baseline 25(OH)D (β active-control: 0.39 kg, p = 0.025), as well as statistical significant interaction effect of treatment by baseline protein (β active-control: 0.38 kg, p = 0.032). Since both interactions were fitted in one model, this indicates that both baseline 25(OH)D concentration as well as baseline protein intake affected the change in aMM by the intervention corrected for each other. Participants in the intervention group with both higher baseline 25(OH)D concentrations and higher baseline protein intakes had a significant increase in aMM (β active-control: 0.59 kg (95% CI: 0.29–0.90), p < 0.001, Fig. 1), while no significant difference was found in the other composite subgroups. Similar results were found for SMI and relative appendicular muscle mass. In a sensitivity analysis, there were similar effects in both male and female participants when analyzed separately (Supplemental Table 1).

4. Discussion

In this post-hoc analysis of the PROVIDE study, a greater muscle mass gain was observed in sarcopenic participants with higher baseline serum 25(OH)D concentrations as well as a higher baseline dietary protein intake in response to a vitamin D and leucine-enriched whey protein supplement. There was, however, no effect modification of baseline 25(OH)D status or protein intake on change in lower-extremity function as measured by chair-stand test. Thus, this indicates that sufficient baseline levels of 25(OH)D

Table 1
Baseline characteristics by baseline 25-hydroxyvitamin D and protein intake subgroups.

	Baseline 25(OH)D concentration (n = 374)			Baseline protein intake (n = 364)		
	<50 nmol/L n = 195	≥50 nmol/L n = 179	P-value	<1.0 g/kg/d n = 200	≥1.0 g/kg/d n = 164	P-value
Demographic, clinical and nutritional characteristics[a]						
Age, years	78.3 (7.1)	77.2 (6.5)	0.134	78.0 (6.6)	77.3 (7.3)	0.293
Sex, female, n (%)[b]	134 (68.7%)	112 (62.6%)	0.231	119 (59.5%)	115 (70.1%)	0.037
Living independently, n (%)[b]	156 (80.0%)	167 (93.3%)	<.001	176 (88.0%)	138 (84.1%)	0.359
Number of co-morbidities[c,d]	4.0 (3.0, 6.0)	4.0 (2.0, 5.0)	0.038	4.0 (3.0, 5.0)	4.0 (3.0, 6.0)	0.718
PASE questionnaire[b]	89.7 (68.9)	111.1 (74.5)	0.004	100.2 (71.1)	102.5 (73.8)	0.770
Geriatric Depression Scale[c,d]	2.0 (1.0, 4.0)	1.0 (0.0, 3.0)	0.005	2.0 (1.0, 3.0)	2.0 (1.0, 3.0)	0.412
MMSE[c,d]	28.5 (27.0, 30.0)	29.0 (28.0, 30.0)	0.023	29.0 (27.0, 30.0)	29.0 (28.0, 30.0)	0.508
MNA score	12.9 (1.5)	13.4 (1.1)	<.001	13.2 (1.4)	13.1 (1.3)	0.563
25(OH)D concentration, nmol/L	34.0 (9.2)	70.6 (17.5)	<.001	49.8 (19.7)	52.9 (26.3)	0.218
Vitamin D intake (μg)	3.1 (4.2)	3.7 (3.7)	0.194	3.0 (3.9)	3.7 (4.0)	0.079
Protein intake, g/kg/day[c,d]	1.0 (0.8, 1.2)	1.0 (0.9, 1.2)	0.366	0.8 (0.7, 0.9)	1.2 (1.1, 1.4)	<.001
Body composition parameters						
Weight, kg	68.6 (11.2)	71.0 (11.2)	0.039	73.1 (10.2)	66.2 (11.4)	<.001
♂	75.9 (10.8)	80.1 (9.3)	0.020	80.3 (9.5)	74.7 (10.7)	0.002
♀	65.3 (9.7)	65.6 (8.3)	0.817	68.3 (7.3)	62.6 (9.7)	<.001
BMI, kg/m[b]	26.0 (2.9)	26.2 (2.5)	0.560	26.7 (2.4)	25.5 (2.8)	<.001
♂	26.2 (2.8)	26.8 (2.2)	0.258	26.8 (2.3)	26.1 (2.8)	0.114
♀	25.9 (2.9)	25.8 (2.5)	0.804	26.6 (2.4)	25.2 (2.8)	<.001
Appendicular Muscle Mass, kg	17.0 (3.6)	18.4 (4.1)	0.001	18.6 (4.0)	16.7 (3.7)	<.001
♂	20.6 (2.8)	22.8 (2.8)	<.001	22.3 (3.0)	20.8 (2.8)	0.009
♀	15.4 (2.6)	15.9 (2.3)	0.118	16.1 (2.3)	15.0 (2.6)	<.001
SMI, aMM/h^2, kg/m^2	6.4 (0.9)	6.7 (0.9)	0.002	6.8 (1.0)	6.4 (0.9)	<.001
♂	7.1 (0.8)	7.6 (0.7)	<.001	7.4 (0.8)	7.2 (0.7)	0.230
♀	6.1 (0.8)	6.3 (0.7)	0.146	6.3 (0.8)	6.1 (0.8)	0.020
Appendicular Muscle Mass/BW, %	24.9 (3.2)	25.9 (3.1)	0.002	25.4 (3.2)	25.3 (3.3)	0.757
♂	27.4 (2.6)	28.4 (2.5)	0.032	27.8 (2.4)	28.1 (2.9)	0.484
♀	23.7 (2.7)	24.5 (2.5)	0.028	23.8 (2.6)	24.1 (2.7)	0.333
Fat Mass, kg (DXA)	25.7 (6.6)	25.5 (5.5)	0.779	27.2 (5.4)	23.8 (6.3)	<.001
♂	23.5 (6.9)	25.4 (5.4)	0.124	25.8 (5.5)	22.5 (6.6)	0.006
♀	26.6 (6.3)	25.6 (5.5)	0.206	28.1 (5.2)	24.3 (6.1)	<.001
Muscle strength and function						
Handgrip strength, kg	19.3 (6.8)	22.3 (8.3)	<.001	21.8 (8.2)	19.8 (6.9)	0.010
♂	25.2 (6.6)	29.2 (7.0)	0.001	28.4 (7.1)	25.2 (6.8)	0.013
♀	16.6 (5.0)	18.1 (5.8)	0.037	17.3 (5.4)	17.4 (5.5)	0.826
SPPB, score	7.2 (1.9)	7.8 (2.0)	0.002	7.5 (1.9)	7.5 (2.0)	0.901
♂	7.2 (2.0)	8.2 (1.8)	0.004	7.7 (1.9)	7.7 (2.1)	0.930
♀	7.2 (1.8)	7.6 (2.0)	0.118	7.3 (1.9)	7.4 (2.0)	0.729
Gait Speed, m/s	0.7 (0.2)	0.8 (0.2)	<.001	0.8 (0.2)	0.7 (0.2)	0.221
♂	0.7 (0.2)	0.9 (0.2)	<.001	0.8 (0.2)	0.8 (0.2)	0.083
♀	0.7 (0.2)	0.8 (0.2)	0.109	0.7 (0.2)	0.7 (0.2)	0.862
Chair-stand time, s[c,d]	17.8 (14.9, 21.4)	16.9 (14.8, 19.8)	0.065	17.2 (15.1, 20.5)	17.2 (14.4, 21.2)	0.862
♂	17.1 (14.8, 21.3)	16.4 (14.6, 18.3)	0.108	16.7 (15.3, 19.3)	16.7 (14.1, 20.6)	0.471
♀	17.9 (14.9, 21.4)	17.2 (14.9, 20.8)	0.324	17.8 (15.1, 20.6)	17.8 (14.5, 21.4)	0.894

[a] Results are presented as mean (SD) with the P-value based on a two-sample t-test unless otherwise stated.
[b] P-value based on a Fisher's exact test.
[c] P-value based on a Mann–Whitney test.
[d] Median (IQR).

and protein intake may be required to increase muscle mass in response to this specific 3-month nutritional intervention.

In observational as well as experimental studies, both vitamin D (status and intake) and dietary protein have been associated with muscle mass, strength and function in older adults. Baseline characterization of the PROVIDE study participants showed that those with higher baseline 25(OH)D had higher muscle mass, handgrip strength, and better physical performance. These cross-sectional findings are in line with other studies where lower or deficient levels of 25(OH)D were associated with lower muscle mass and lower extremity function, higher risk for falls and fractures, and nursing home admissions [3,16–19].

Intervention studies combining adequate levels of vitamin D and high quality protein in older populations are scarce. We observed that higher baseline vitamin D levels may be required to lead to a gain in muscle mass in response to a specific nutritional intervention. This effect was independent of the level of physical activity. A recent meta-analysis found an overall positive effect of vitamin D supplementation on muscle strength and function, but not on muscle mass [6]. Based on the present results it could be suggested that a beneficial effect of vitamin D supplementation may be dependent on both the dose given and the baseline 25(OH)D concentration. Older adults with deficient baseline concentrations may need an even higher dose of vitamin D or longer supplementation periods to achieve the desirable serum 25(OH)D concentrations (>50 nmol/L [12], or even 75–100 nmol/L [20]) and effects on muscle outcomes.

Similarly, a higher baseline protein intake (>1.0 g/kg BW/d) led to a greater increase in muscle mass in response to the supplementation. At baseline, however, both men and women with a lower relative protein intake (<1.0 g/kg BW/d) had more muscle mass and stronger handgrip strength. This is counter to what is frequently described in literature, where insufficient daily protein intake is associated with low muscle mass and strength [21–24].

Table 2
Effect modification of baseline 25-hydroxyvitamin D and protein intake on change in muscle parameters.

	Baseline 25(OH)D concentration[a]			Baseline protein intake[d]		
	<50 nmol/L β active-control[b] (95% CI), p-value	≥50 nmol/L β active-control[c] (95% CI), p-value	Between-subgroup P-value	<1.0 g/kg/d β active-control[e] (95% CI), p-value	≥1.0 g/kg/d β active-control[f] (95% CI), p-value	Between-subgroup P-value
Change in appendicular muscle mass (kg)	−0.01 (−0.24, 0.22), p = 0.94	0.35 (0.11, 0.60), p = 0.004	0.034	0.00 (−0.23, 0.23), p = 0.97	0.42 (0.16, 0.67), p = 0.001	0.020
Change in appendicular muscle mass/height^2 (kg/m^2)	−0.00 (−0.09, 0.08), p = 0.95	0.12 (0.03, 0.21), p = 0.007	0.048	0.00 (−0.09, 0.09), p = 1.00	0.15 (0.06, 0.24), p = 0.002	0.021
Change in appendicular muscle mass/body weight (%)	−0.05 (−0.39, 0.28), p = 0.75	0.51 (0.16, 0.86), p = 0.004	0.023	0.01 (−0.33, 0.34), p = 0.75	0.56 (0.19, 0.92), p = 0.003	0.029
Change in chair-stand time (sec)[g]	*−1.50 (−3.07, 0.07)*, p = 0.388	*−0.52 (−2.22, 1.19)*, p = 0.278	0.850	*−1.67 (−2.93, −0.41)*, p = 0.061	*−0.70 (−2.45, 1.06)*, p = 0.564	0.440

[a] n = 256 and n = 250 participants with complete data for both baseline 25(OH)D and muscle mass measures and chair stand test respectively.
[b] n = 64 active vs. n = 70 control in muscle mass measures and n = 65 active vs. n = 66 control in chair stand test.
[c] n = 60 active vs. n = 62 control in muscle mass measures and n = 57 active vs. n = 62 control in chair stand test.
[d] n = 249 and n = 241 participants with complete data for both baseline protein intake and muscle mass measures and chair stand test respectively.
[e] n = 64 active vs. n = 70 control in muscle mass measures and n = 67 active vs. n = 72 control in chair stand test.
[f] n = 55 active vs. n = 60 control in muscle mass measures and n = 49 active vs. n = 53 control in chair stand test.
[g] Chair stand time was not normally distributed; therefore p-values are from the ANCOVA model using log-transformed chair stand time. To avoid complex interpretation with log transformed values, the mean and 95% CI for intervention − control for changes in chair stand time based on a t-test using untransformed values are presented in italics.

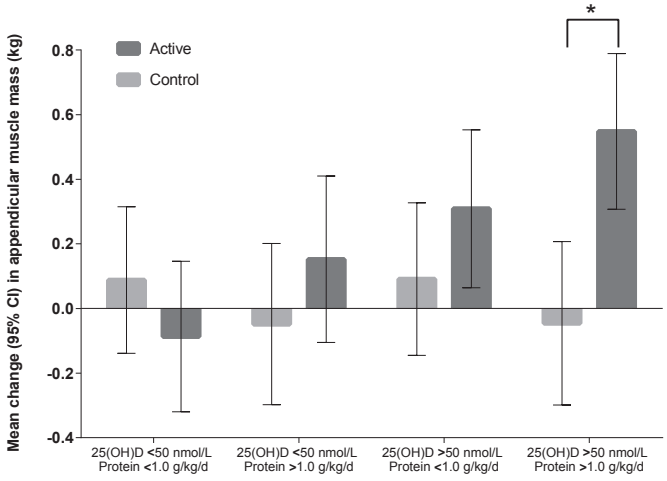

Fig. 1. Effect modification by combined baseline 25(OH)D and baseline protein intake subgroups on mean change (95% CI) in appendicular muscle mass (kg) between active and control in four subgroups (<50 nmol/L 25(OH)D, <1.0 g/kg/d protein, active n = 37 and control n = 37; <50 nmol/L 25(OH)D, ≥1.0 g/kg/d protein, n = 26 and n = 31; ≥50 nmol/L 25(OH)D, <1.0 g/kg/d protein, n = 28 and n = 32; ≥50 nmol/L 25(OH)D, ≥1.0 g/k/d, n = 27 and n = 28).

We found that a higher absolute protein intake (expressed in g/d and not as ratio g/kg BW/d), was significantly associated with higher skeletal muscle mass, strength and function.

In addition to insufficient intake, older adults often display a blunted muscle protein synthetic response to dietary protein ingestion [25–27] and to insulin [28], which is known as anabolic resistance. Physical activity in addition to the optimal type and amount of protein per meal [4,5] and other nutritional factors such as vitamin D might help to overcome the anabolic threshold in sarcopenic older adults. Since vitamin D deficiency is associated with reduced muscle mass and insulin resistance among older adults [16,29], vitamin D might play a role in anabolic stimulation induced by amino acids like leucine and insulin. In a recent report [30], vitamin D acted synergistically with leucine and insulin to stimulate muscle protein synthesis, likely through sensitizing the anabolic pathways induced by insulin and leucine. These data emphasize that nutritional interventions combining vitamin D and amino acid supplementation might be a promising strategy targeting muscle preservation, especially in conditions as in sarcopenia where vitamin D deficiency often coincides with a decreased response to amino acids [26], and insulin [28].

For both vitamin D and protein, recent recommendations encourage increased intakes for both healthy older adults and older patients. The optimal diet of healthy older adults should contain >1.0 g protein/kg body weight/day and up to 1.5 g/kg/d for older patients, with at least 25–30 g of high-quality protein at each main meal [14,31,32]. This is in addition to adequate vitamin D intake at 800 IU/day to maintain serum 25(OH)D levels >50 nmol/L [11]. Recently, the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO) presented similar recommendations for maintaining musculoskeletal health [13]. The cut-offs of these recommendations (1.0 g protein/kg/d and 50 nmol/L 25(OH)D) may thus be required in order to surpass the threshold of anabolic stimulation and thus improved muscle mass gain in the older population. Participants with a lower protein intake or low 25(OH)D status may require a prolonged period of supplementation to improve protein and 25(OH)D status.

4.1. Strengths and limitations

The relatively large sample size provided the statistical power to detect small differences in muscle mass gain between the subgroups. In addition, we standardized the analysis of raw DXA data centrally to provide uniform and reliable body composition data. The multi-centre nature of this study improves the generalizability of our findings, especially given the variability in baseline vitamin D concentrations by country.

The following limitation must also be discussed to give context to the study strengths. Though changes in muscle mass, in particular gain, is most often achieved with resistance exercise [4,5,33] we did not combine the intervention with exercise. We were

interested in investigating the effect of nutritional supplementation alone on measures of sarcopenia to act as a reasonable facsimile for times when exercise is neither possible nor feasible (e.g. post-surgery). However, we acknowledge that exercise in combination with adequate nutritional intake is the clinical gold standard for managing sarcopenia. Furthermore, from the current study design we cannot conclude whether a specific protein source and/or specific amino acids, or merely the total protein intake resulted in what we observed.

5. Conclusion

Sarcopenic participants may need serum 25(OH)D concentrations exceeding 50 nmol/L and a fairly high dietary protein intake (>1 g/kg body weight/day) in order to experience meaningful muscle mass gain from a vitamin D and protein supplement in long term interventions. This suggests that cut-offs in current recommendations for vitamin D status and dietary protein intake could be considered the “minimum” for adults with sarcopenia to respond adequately to nutrition strategies aimed at attenuating muscle loss. Nutritional interventions combining adequate amounts of protein and vitamin D, ideally in combination with physical activity, are promising strategies to attenuate sarcopenia development, which can contribute to prolonged independence and vitality with age.

Conflict of interest

Verlaan and Wijers are empolyees of Nutricia Research, Nutricia Advanced Medical Nutrition.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.clnu.2017.01.005.

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